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Lymphoproliferative Disease in the Rectum 4 Years After Local Mesenchymal Stromal Cell Therapy for Refractory Perianal Crohn's Fistulas: A Case Report

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Abstract

Mesenchymal stromal cell [MSC] therapy is a new treatment for perianal fistulas in Crohn's disease. Although MSC therapy shows a favourable safety profile, long-term safety data are limited. We detected an Epstein Barr virus [EBV]-associated B cell lymphoproliferative lesion in the rectum of a patient 4 years after local administration of MSCs for his perianal fistulas. To investigate whether MSC therapy contributed to the development of this lymphoproliferative disease, we analyzed the possibility of EBV transfer via the MSC product and the persistence of MSCs in the lymphoproliferative lesion using short tandem repeat analysis.

Key Words: perianal fistulas; Crohn's disease; mesenchymal stromal cells; lymphoproliferative disease.

1. Introduction

In 2014, we conducted a dose-finding placebo-controlled clinical trial in which allogeneic bone marrow–derived mesenchymal stromal cells [MSCs] were administered locally to Crohn's disease [CD] patients with refractory perianal fistulas.[1] Fistula healing at week 6 was observed in 80% of the patients treated with 30×10^6 MSCs, compared with 17% in the placebo group. Four years later, we invited 20 study patients for long-term follow-up and performed endoscopy of the rectum and pelvic MRI. Here, we report a case of lymphoproliferative disease [LPD] in the rectum detected 4 years after MSC therapy for perianal fistulizing CD.

2. Case report

One of the patients treated with a local injection of 30×10^6 MSCs was a 45-year-old man. In 2008, he was diagnosed with CD of the

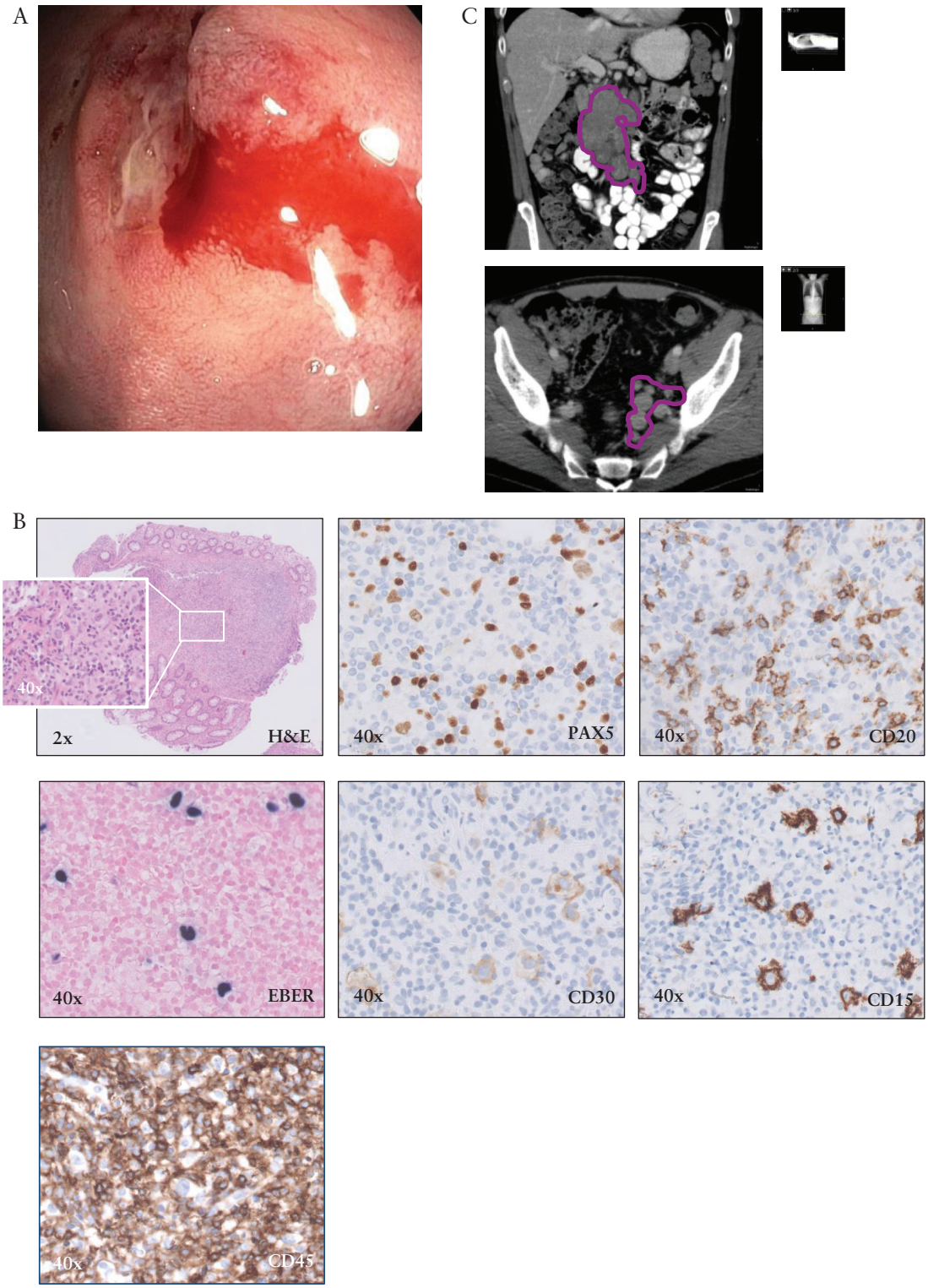

Figure 1. LPD in the rectum of patient previously treated with local MSC therapy. [A] Ulcer in the rectum with a raised edge next to the scar of the old fistula opening after biopsy. [B] Routine immunohistochemistry performed on biopsy from the lesion using the classical markers PAX5, CD20, CD30, CD15, and CD45. *In situ* hybridisation [dark staining] for Epstein-Barr encoding region [EBER] to detect EBV in the lesion. [C] MRI-scan of the abdomen within the coronal plane para-aortal multiple lymphoproliferative processes around the a. mesenterica inferior and in the axial plane multiple lymphoproliferative processes surrounding the a. and v. iliaca interna.

colon, predominantly left-sided. In 2012, he developed a trans-sphincteric fistula with one internal and two external openings. He was treated with prednisone [2008], azathioprine [2008–2012], adalimumab [2011–2012], and methotrexate [2012–2017]. Since

MSC therapy in 2014, his fistula was closed. In 2016, he started with vedolizumab, which he has continued since, because of a luminal exacerbation. Surveillance endoscopy in the summer of 2017 revealed no abnormalities and normal pathology of biopsies.

At the long-term follow-up visit, 4 years after MSC therapy, the patient reported no complaints and had a normal faecal calprotectin of 46 µg/g. The scheduled MRI at this visit showed fibrosis of the distal part of the fistula tract. The cranial part of the fistula was no longer visible. During scheduled proctoscopy, an ulcer next to the scar of the old fistula opening was seen with a raised edge. The diameter was ~10 mm [Figure 1A]. Biopsies were obtained and histology revealed the presence of an Epstein-Barr virus [EBV]-associated B cell LPD [Figure 1B]. The lesion contained a combination of small lymphocytes and blastoid cells, positive for EBV-encoded RNA and expressing PAX-5, CD20, CD15, and to a lesser extent CD30 [Figure 1B]. Laboratory tests showed a low level of EBV virus–specific DNA in his serum. Of note, our patient was IgG EBV–positive, but IgM EBV–negative at the time of MSC therapy in 2014. A subsequent CT scan of the abdomen and thorax showed the presence of extensive lymphadenopathy [Figure 1C]. The patient was diagnosed with Lugano Stage IV EBV-associated B cell LPD and was treated successfully with chemotherapy.

Retrospective analysis of the biopsies of the rectum taken in 2014 excluded the presence of LPD prior to MSC administration, because no rectum localization of EBV could be detected using EBV-encoded RNA *in situ* hybridization. Next, we investigated a potential role for MSC therapy in the development of this LPD, considering the following options: 1. Transfer of LPD via the MSC Drug Product; 2. Transfer of EBV via the MSC Drug Product; 3. Tumorigenicity due to persisting local allogeneic MSCs. Regarding the first option, theoretically, an LPD present in the MSC Drug Product could be transferred to the patient. However, no $CD45^+$ cells, and thereby B cells, were detected in the MSC Drug Product [10 000 cells screened]. This was in accordance with the release criteria for MSC Drug Products generated for this study [≤1% $CD45^+$ cells]. Additionally, all treating physicians of all other patients who received an MSC Drug Product obtained from the same donor [$n = 9$, Table 1] were contacted about the potential presence of LPDs in their patients. No LPDs were reported in the other patients treated with MSCs obtained from this donor bone marrow. Furthermore, at the time of bone marrow donation, the MSC donor was in good health, without any signs of LPD.

The MSC-donor was positive for IgG EBV at the time of bone marrow donation. To investigate a potential transfer of EBV-virus to the recipient using MSCs as a vehicle, the MSCs that were infused in the patient and two other MSC Drug Products obtained from donors positive for IgG EBV at the time of bone marrow donation were tested for the presence of EBV-specific DNA using PCR. No EBV-DNA was detected in the MSCs infused in this patient; nor was there any in the other Drug Products that were tested.

To investigate whether the injected MSCs were still present in the lesion in the rectum, flow cytometry sorting was used to separate cells in rectum biopsies into $CD45^+CD19^{dim/+}$ and $CD45^-CD15^{dim/+}$ cells [Figure 2A, panel A: representing healthy and malignant B-cells], and a $CD45^-CD15^-$ fraction [Figure 2A, panel B: representing the stromal–epithelial population, which would include the allogeneic MSCs, if still present]. Standard short tandem repeat [STR] analysis was used to examine the presence of genomic material of the MSC donor in both sorted cell fractions, as well as in 20 000 unfractionated cells obtained from the same biopsy. Although cell counts and DNA recovery was low, some STR regions on nuclear DNA could be evaluated successfully. All fractions exclusively showed the STR profile of the patient. Together, these data indicated that no allogeneic MSCs were present in the biopsied rectal lymphoma [Figure 2B].

3. Discussion

In this report we present a patient diagnosed with LPD in the rectum 4 years after local MSC therapy for perianal fistulizing CD. We hypothesized that the occurrence of LPD in this patient could be explained by his underlying IBD, prolonged use of immunosuppressive medication, including methotrexate, vedolizumab, anti-tumor necrosis factor [TNF]-therapy, and azathioprine, or local MSC infusion.

Studies on the risk of LPD in patients with IBD generated conflicting results. In contrast to rheumatoid arthritis, IBD itself does not seem to cause a statistically significant increased risk of LPD.[2–6] However, IBD patients using immunosuppressive medication have an increased risk of LPD.[2] These LPDs are often extranodal and are the result of EBV-driven processes, although other infectious agents could also be involved.[2,7] Our patient used several immunosuppressive therapies in the last 10 years, and each of these treatments is associated with an increased risk of developing LPD. Until 5 months before LPD diagnosis, the patient was using methotrexate as an immunosuppressive therapy. Methotrexate-associated LPD is recognized as an independent entity and is characterized by the presence of EBV virus in the lymphoma tissue. In most patients, a regression of the LPD is seen after discontinuation of methotrexate.[8] At the time of LPD diagnosis, the patient was treated with vedolizumab. As vedolizumab has only been available for a few years, extensive data on the long-term safety profile are not available yet.[9] Furthermore, our patient had used azathioprine for 4 years and anti-TNF therapy in the past. Although, current use of thiopurines in IBD is associated with an increased risk of LPD, conflicting data are available about the risk of LPD after withdrawing thiopurines and anti-TNF

Table 1. Overview of other patients treated with MSCs from the same donor as our patient.

	Age at time of treatment (years)	Gender	Indication for MSC therapy	Time of follow-up [months]	LPD reported
1	33	V	CD fistula, MSC-F trial	48	No
2	30	V	CD fistula, MSC-F trial	48	No
3	22	M	CD fistula, MSC-F trial	48	No
4	9	M	GvHD, Study P05.089	5.5	No
5	10	V	GvHD, Study P05.089	<1	No
6	15	V	GvHD, Study P05.089	10	No
7	42	M	GvHD, hospital exemption	50	No
8	39	V	GvHD, hospital exemption	3.5	No

CD, Crohn's disease; GvHD, steroid refractory graft-versus-host disease; LPD, lymphoproliferative disease; MSC-F trial,[1] Study P05.089 [LUMC].[19]

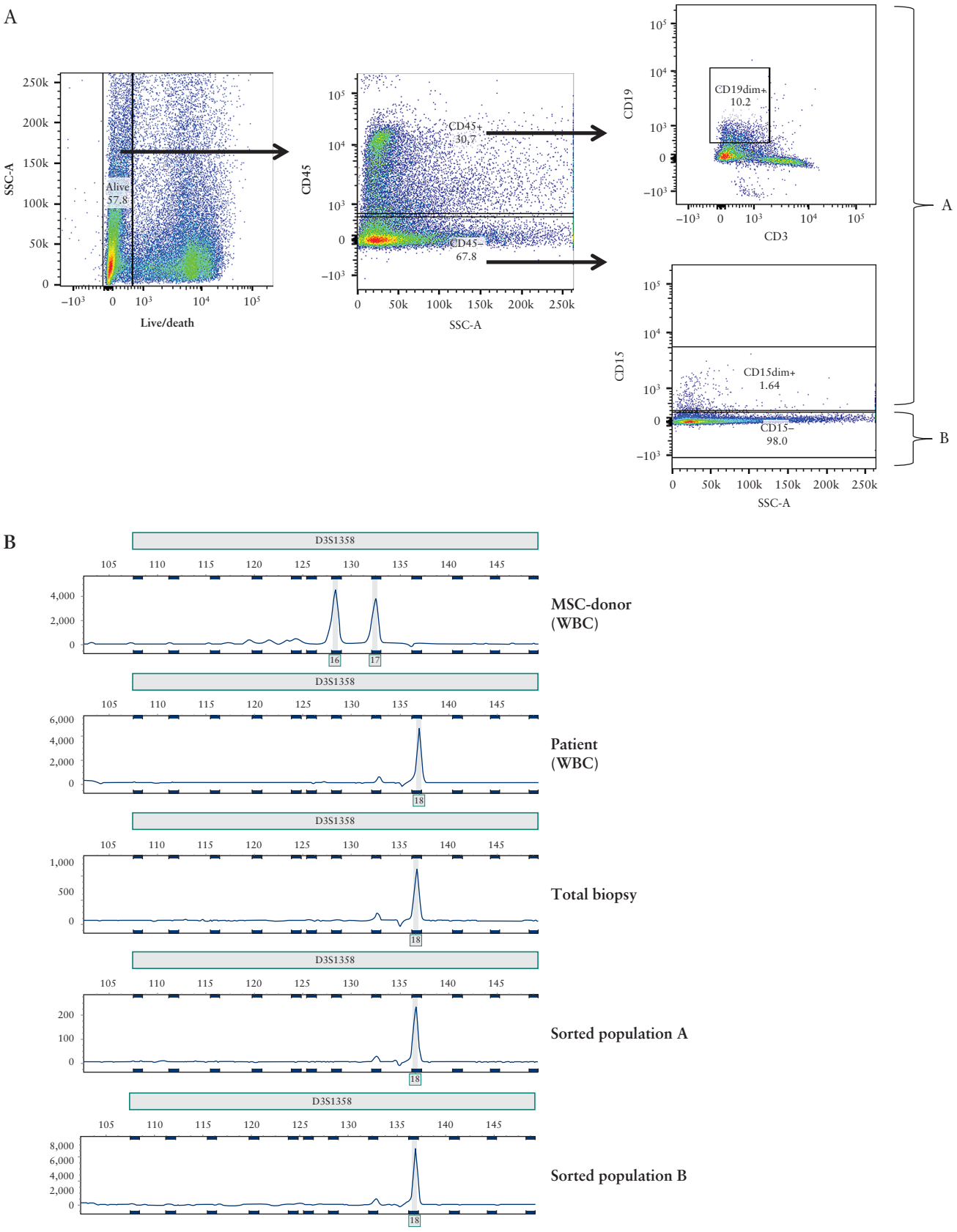

Figure 2. Analysis of the presence of allogeneic MSCs in the LPD in the rectum. [A] Flow cytometry sorting gating stragety for population [A] $CD45^{+}CD19^{dim/+}$ and $CD45\text{-}CD15^{dim/+}$ cells and [B] $CD45^{-}CD15^{-}$ cells. [B] Peak profile of short tandem repeat marker D3S1358 in the DNA of the MSC-donor and patient, obtained from white blood cells [WBCs], and in the DNA from the rectum biopsy and the two sorted populations [A and B] out of the biopsy.

treatment.[2,10–13] Therefore, we cannot exclude the possibility that the patient developed LPD as a result of immunosuppressive therapy other than a single MSC gift in 2014.

As MSCs have immunomodulatory properties and may have served as a vehicle for EBV-transfer, we investigated the potential role of MSC therapy in this LPD. We excluded the possibility that the EBV virus was transferred via the infused MSC or by contaminating EBV-positive B cells present in the MSC Drug Product. Furthermore, we did not detect any allogeneic MSCs or DNA specific for the MSC donor in the lesion in the rectum. No data are available on the risk of LPD in patients who have received MSC therapy. Overall, intravenous MSC therapy does not seem to be associated with significant immunosuppression in immunocompetent individuals.[14] However, an additional immunosuppressive effect in patients already on immunosuppressive medication cannot be excluded. In 52 renal transplant patients treated in our centre with intravenous MSCs, no LPD was reported [follow up 6 months–4 years]. However, in the literature, two cases of EBV-associated LPD after systemic MSC treatment are described in patients with Graft-versus-Host Disease,[15,16] a severely immunocompromised patient population. No data are available on LPD following the local injection of MSCs.

Taken together, it is highly unlikely that this EBV-associated LPD was directly related to MSC therapy, but rather it is likely to have been the result of prolonged immunosuppressive therapy. However, we cannot exclude the possibility of additional local immunosuppression by MSC therapy, which subsequently may drive proliferation of tissue-resident EBV-infected cells. Darvadstrocel,[17] a product containing MSCs isolated from adipose tissue, is now approved in Europe as a treatment for complex perianal fistulas in patients with Crohn's disease. One year follow-up data showed a favorable safety profile of this product.[18] This case report shows that more long-term reports on MSC therapy in perianal fistulas from clinical trials and daily practice are needed to evaluate the complete safety profile of topical MSC therapy.

Author Contributions

MCB: study design, acquisition of data, data analysis, and writing up first draft of the paper; AGSH: study design, acquisition of data, data analysis, and revising the article; MP: study design, acquisition of data, and revising the article; IM: inclusion of patients, and revising the article; ACS: acquisition of data, and data analysis; PMJ: acquisition of data, and data analysis; HWV: study design, and revising the article; GD: acquisition of data, revising the article; LEMO: study design, acquisition of data, revising the article; AEMJ: study design, revising the article.

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Conflict of interest

The authors declare that they have no conflicts to declare.

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Written informed consent prior to submission was obtained from the patient.

Supplementary Data

Supplementary data are available at *ECCO-JCC* online.

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